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Traversing the Barrier: A Journey of Insulin from Vascular Lumen into Skeletal Muscle

Hong Wang*

Division of Endocrinology and Metabolism, Department of Medicine, University of Virginia Health System, Charlottesville, VA 22908, USA

Plasma insulin must traverse the vascular endothelium to reach its major sites of action on myocytes and adipocytes. In study simultaneously measuring plasma and lymphatic insulin concentrations in normal, conscious dogs during euglycemic insulin clamps it was found that the steady-state plasma insulin concentration was consistently higher than lymph with a rough ratio of 3:2 during the basal period. In addition, while plasma insulin concentration rose quickly to reach the steady-state during the insulin clamp, the lymph insulin concentration rose very slowly indicating a barrier function of the vascular endothelium. Most importantly, this study showed that the dynamics of glucose disposal correlated very strikingly with the insulin concentration in lymph but not plasma, suggesting that trans-capillary insulin transport is a rate limiting step for peripheral insulin action [1]. Consistent with this, direct injection of insulin into canine skeletal muscle in vivo was recently noted to significantly hasten the onset of muscle glucose utilization compared with intravenously delivered insulin [2]. Multiple other studies also support such an important role of vascular endothelium during insulin clamp by measurement of the insulin concentration within skeletal muscle interstitium in humans and animals using either lymphatic sampling or microdialysis methods [3-7]. Results obtained using either method indicate that even after several hours of steady state hyperinsulinemia, muscle interstitial insulin concentration is only 40-50% of that in plasma and the time course for insulin-mediated glucose disposal during the euglycemic clamp correlates strongly with interstitial but not plasma insulin concentrations. Based on these findings, it has been estimated that slow trans-endothelial insulin transport may account for 30-40% of insulin resistance seen with human obesity or type-2 diabetes [3,8,9].

Current evidence indicates that insulin Trans Endothelial Transport (TET) is mediated by transporting caveolae that contain or associate with multiple structural and signaling molecules including caveolin-1, Insulin Receptor (IR), IGF-1R, dynamin 2, actin filaments and eNOS [10-18]. Early studies demonstrated that vascular Endothelial Cells (ECs) express IRs and that insulin TET is saturable and mediated by IRs [10,19,20]. Subsequent in vivo studies also reported that at physiological insulin concentrations insulin TET into human skeletal muscle interstitium is saturable [21,22]. However, several in vivo studies were unable to observe the saturation when supraphysiological insulin doses were applied during an insulin clamp [23,24]. In 2006, we employed a different approach using confocal microscopy and serial muscle biopsies and found that intravenously infused Fluorescein Isothiocyanate (FITC)-labeled insulin rapidly localized within the vascular ECs of skeletal muscle but not in the intercellular clefts in vivo [18]. Given that vascular ECs also possess IGF-1 receptors and IGF-1Rs are ~10x more abundant than IRs with a much lower affinity for insulin's binding we found that both IGF-1 peptide and a neutralizing antibody against IGF-1R significantly inhibited insulin uptake and TET when a pharmacologic insulin concentration (50nM) was used [18,25,26]. This has provided an alternative explanation for the seemingly conflicting data regarding the saturability of insulin transport into muscle, i.e. that at physiological insulin concentrations insulin TET is mediated predominantly by IRs but at supraphysiologic insulin concentrations both IR and IGF-1R (and IR/IGF-1R hybrid receptors) contribute to insulin TET [27,28]. Caveolin-1,a 21-kDa integral membrane protein required for caveolae formation is required

for receptor-mediated albumin uptake by vascular ECs [29-31]. We have previously reported that antibodies against IR and caveolin-1 mutually co-immunoprecipitate one another from ECs and others have reported that IR binds to caveolin-1 scaffolding domain through its caveolin-1 binding domain [18,32,33]. Moreover, a recent electron microscopic immunocytochemical study has convincingly shown that IRs are present throughout in the plasma membrane but are particularly concentrated at the neck of caveolae in 3T3-L1 adipocytes [34]. IGF-1Rs appear to have similar lipid raft/caveolae localization in the plasma membrane [35]. We have reported that knockdown of caveolin-1 expression in bAECs using specific caveolin-1 siRNA reduces caveolin-1 mRNA and protein expression by ~70%, and reduces FITC-insulin uptake by 67%, whereas over-expression of caveolin-1 increases insulin uptake. In addition, knockdown of caveolin-1 significantly reduces both insulin receptor protein level and insulin-stimulated Akt1 phosphorylation [13]. Dynamin-2 is a large GTPase that regulates caveolae-mediated endocytosis of cholera toxin and albumin by promoting the separation of caveolae from the plasma membrane via GTP hydrolysis. We have reported that dynamin-2 is also required for caveolae-mediated insulin uptake. Either inhibition of dynamin-2 function (with Dynasore) or siRNA knockdown of dynamin-2 inhibits vascular EC insulin uptake [12]. Insulin has been reported to induce rapid cortical actin filament remodeling in a variety of cell types including vascular ECs. This remodeling has been found to correlate to an increased transport of nutrients such as amino acids and glucose and caveolae-mediated macromolecules endocytosis [36-40]. We have recently reported that insulin-induced cortical actin filament remodeling in ECs is required for caveolae-mediated insulin's uptake and TET in a PI3-kinase and plasma membrane lipid rafts dependent fashion [14]. We have also reported that insulin act on vascular ECs to facilitate its own uptake and TET through multiple intracellular signaling pathways including PI-3 kinase-Akt, MAP kinase and Src pathways [14,15]. Very recently, we have reported that eNOS and its activity play a critical role in regulation of insulin uptake and TET as inhibition of eNOS activity completely eliminates EC insulin uptake [16]. Taken together, our studies indicate that there is the vesicular transcytotic machinery in vascular ECs that governs insulin uptake and movement through vascular ECs.

This endothelial barrier function is particularly true for skeletal muscle, a major site of fuel use, where its continuous vascular endothelium has well-developed junctional structures and abundant caveolae that provides a relatively tight diffusional barrier. This is in stark contrast to the discontinuous endothelium with gaps between

*Corresponding author: Hong Wang, Department of Medicine, University of Virginia, Box 801410, Charlottesville, VA 22908, USA, Tel: 434-924-1265; Fax: 434-924-1284; E-mail: Hw8t@virginia.edu

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vascular ECs in liver. Muscle's tight endothelium has constituted the structural basis for a strong argument that the transit of insulin from the vascular lumen to the interstitial compartment within skeletal muscle is rate limiting for insulin's metabolic action [27]. In an early study comparing the kinetics of insulin action on peripheral glucose disposal during the insulin clamp between lean and obese subjects, it was found that although hepatic glucose output was suppressed rapidly ($t\frac{1}{2}$ ~ 20 min) and did not differ between normal and insulin resistant subjects, the $t\frac{1}{2}$ for stimulation of whole body glucose disposal was quicker (~44 min) in lean adults than obese insulin resistant subjects (~74 min, p<0.001) indicating a delay in insulin delivery into the muscle interstitium. Indeed, this rate-limiting step for peripheral insulin action is delayed in insulin-resistant obese subjects [3,9,41,42].

In summary, current evidence clearly indicates that insulin TET is a transcellular process that is governed by endothelial molecular transcytotic machinery involving insulin receptor binding, activation of EC insulin signaling and membrane trafficking via caveolae. This process plays a critical role in regulation of insulin delivery into and action in the peripheral tissues under both physiological and pathophysiological conditions. Under pathophysiological conditions such as insulin resistance, obesity and type 2 diabetes, this process is significantly delayed or impaired. Better understanding complex regulatory processes and molecular mechanisms of insulin TET under a variety of disease conditions may suggest new therapeutic strategies and offer opportunities to find new intervention sites so that it will improve the treatment for insulin resistance, obesity and type 2 diabetes.

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Page 2 of 3

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Page 3 of 3

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